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Changes in the biochemical composition of the early larval stages of the brine shrimp, Artemia salina *L.*

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Abstract

The dried cysts of the brine shrimp *Artemia salina* are used all over the world as a most convenient source of live crustacean nauplii which are indispensable for the larval stages of many fishes and crustaceans.

With the expansion of mariculture, the demand for the resting eggs of *Artemia* has greatly increased and at certain moments of the year it exceeds the offer. Hence it is even more regrettable that in most mariculture farms a considerable wastage of this precious live food occurs: the nauplii are usually hatched in uncontrolled conditions, and the instar stage at which the *Artemia* larvae are offered to the predator is not considered and varies from one experiment to another.

From fundamental research on the hatching and molting rate of *Artemia* larvae it appears that the water temperature has an important influence on the latter two processes. The biochemical data reported in this paper, reveal drastic changes in the dry- and ash weight and in the caloric- and lipid content of *Artemia* nauplii when they molt from the 1st into the 2nd and 3rd instar stages.

In order to keep the energetic value as high as possible, it is clear that the cysts should be hatched under strictly controlled conditions and should be fed to the larval fishes or crustaceans as soon as possible after hatching.

Introduction

The brine shrimp *Artemia salina* L. which is widely used as food for fishes, decapod and cephalopod larvae, is in fact the only organism on which about 99 % of the aquaculture farmers have relied for the last decades (Sorgeloos and Persoone, 1975).

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The principal reason why brine shrimp nauplii are so widely used for mariculture purposes is undoubtedly that they can be obtained from an (apparently) inert source, namely dried eggs which only need to be immersed in seawater to produce nauplii within 24 hr.

According to Helfrich (1973), the yearly consumption of *Artemia* cysts harvested in nature, approximates 25 tons. With the expansion of aquaculture, the demand for cysts is markedly increasing and at certain periods of the year already exceeds the supply. Despite this critical situation, the cysts of *Artemia* are in many cases not optimally used : the hatching of the larvae is carried out in uncontrolled conditions and the developmental stage at which the larvae are offered to the predator is never controlled.

It is clear that the major factor for an optimal utilization of this live food is the nutritional value of the larvae, which are fed to the fish or crustacean larvae. As a first step towards this goal, we compared in the present study, the dry weight, the caloric value, the lipid content and the fatty acid composition of the early larval stages of *Artemia salina*.

Materials and methods

All experiments were carried out with *Artemia salina* cysts originating from San Francisco Bay (California, U.S.A.) and with artificial seawater of 35 ‰ salinity, prepared following the formula of Dietrich and Kalle (1963).

Hatching of the cysts

The hatching of the nauplii was carried out in cylindrical-conical glass tubes containing 600 ml of seawater and 4 g of cysts (Sorgeloos and Persoone, 1975). The in-

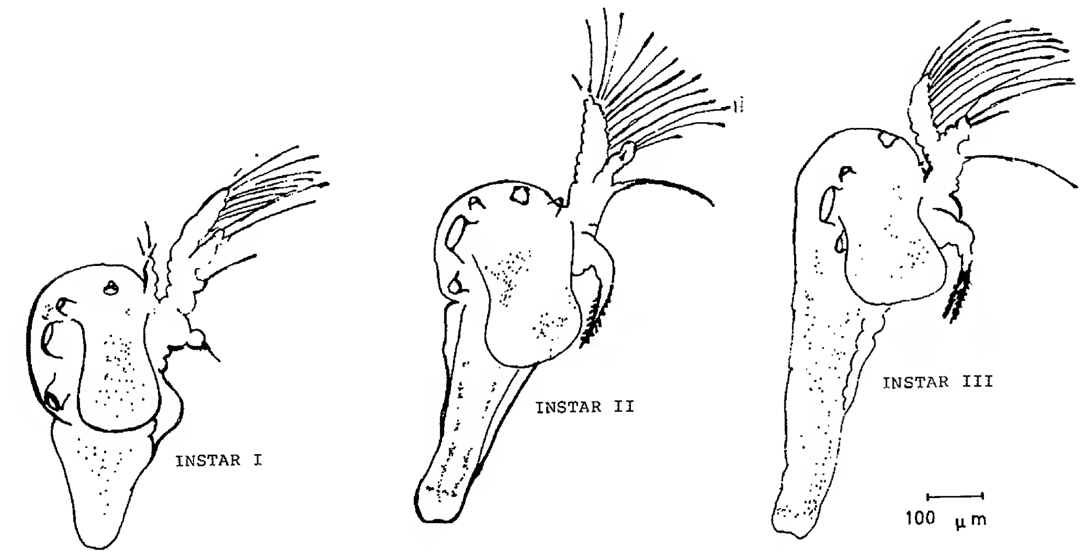

FIG. 1. Morphological characteristics of 1st, 2nd, and 3rd instar nauplii of *Artemia salina* (after Hentschel, 1968).

cubation temperature was 28 ± 0.5 °C. After being immersed for 18 hr., the hatched larvae were harvested and cleared from the hatching debris by means of the separator box of Persoone and Sorgeloos (1972).

The larval instar stage was then determined following the morphological characterisations described by d'Agostino (1965) and Hentschel (1968) (Fig. 1).

Immediately after hatching and separation all the larvae were in the instar I stage.

Half of the nauplii was used directly for biochemical analysis, the rest was transferred to two serum bottles containing each 2 l of seawater. The bottles were hung upside down and continuously aerated by a slight air-bubbling at the bottom.

After 24 hr of incubation at 28 °C the larvae had molted into the 2nd instar stage and part of them already into the 3rd instar stage. Before collecting the animals for biochemical analysis the exuviae and the dead larvae were eliminated by separation with the separator box.

The following determinations were performed on the two batches of respectively instar I, and instar II and III larvae.

Individual dry weight

A total of 10 samples of 200 larvae each, were counted with an optical larvae-counter (precision ± 1 % ; Van Outryve and Sorgeloos, 1975) and filtered on small copper grid filters (diameter 1 cm ; pore size 70 µm). The filters, containing the larvae, were thoroughly washed with filtered deionized water and oven dried at 60 °C for 10-20 hr (Lovegrove, 1966).

In previous experiments it was determined that the weight of the copper grids did not change during the tests.

In order to determine the residual water content of the oven-dried samples, a Karl-Fisher water analysis was carried out under a nitrogen flow.

Ash content

Oven dried samples were incinerated for 4 hr at 550 °C. After cooling for 30 min in a dessicator under vacuum, the ash was weighted on a Cahn electrobalance (Model 4100).

Caloric content

Samples of approximately 20 mg dry weight were compressed into pellets and burned in the microbomb calorimeter of Phillipson (1964). The rough caloric values obtained were reconverted to calories per gram ash-free dry weight.

Total lipid content

Freeze-dried samples were extracted with a 2:1 chloroform:methanol mixture and purified according to a modification of the method of Folch (Bligh and Dyer, 1957). Determination of the lipid content was carried out by gravimetric as well as colorimetric methods. For the colorimetric determination the sulfophosphovanilline reaction was used, with cholesterol as internal standard.

Fatty acid composition

The qualitative and the quantitative fatty acid composition was analyzed by gaschromatography.

Freeze-dried brine shrimp larvae were esterified with a 5 % sulfuric acid in methanol solution.

After 17 hr the methylesters were extracted with petroleum ether, washed with a saturated sodiumchloride solution and dried over magnesium sulfate. The organic solvens was evaporated at 30 °C under a nitrogen flow.

The gaschromatographic analysis was carried out with a Carlo Erba Fractovap 2300, equipped with an F.I.D. detector and temperature programmed from 90-200 °C at 8 °C/min. A 4 m column packed with 5 % EGSSX on gaschrom Q (80-100 mesh) was used.

Results

The results obtained for the different parallels of the replicate series A through D are summarized in Table I.

For each type of analysis the average and the coefficient of variation were calculated per series. The averages for the four series and the procentual increase or decrease of the biochemical components in the 2nd and 3rd instar batch as compared to the first instar sample are given in Table II.

The t- and F-tests revealed statistically significant differences at the $P < 0.05$ level in the dry- and the ash weight between the first and the two following larval stages.

The individual dry weight of a freshly hatched San Francisco Bay *Artemia* nauplius decreased with 20 % within a period of 24 hr at 28 °C.

As the larvae increase significantly in length when molting from the 1st into the 2nd and 3rd instar stages (Sorgeloos, 1975), the ratio of carapax to organic matter increases ; our analyses reveal that the ash content increases from 6 % in the 1st instar up to 11,8 % in the 2nd and 3rd larval stages. As a consequence, the individual ash free dry weight decreases with 24 %.

Within the 24 hr following hatching, the caloric content of the organic fraction of the nauplii decreased with 4 %, which means that during this period the ratio of energy-rich to energy-poor substances decreases.

Calculated at the individual level, the caloric content decreased with 27 % from the 1st to the 2nd, and 3rd instar stages. The drop in the lipid content averaged 26 %, which corresponds to a weight decrease of 0.17 µg per individual.

When expressed in calories, this decrease of the lipid content approximated 1.6 µcal per individual. This decrease in lipids represents 60 % of the total decrease of the energetic content of the brine shrimp, during the first 28 hr of its life.

The gas chromatographic analysis revealed that despite a decrease of 26 % in the total concentration of fatty acids, their relative proportions remained almost unchanged (Table III).

TABLE I

Detailed results of the biochemical analysis of *Artemia* nauplii in four replicate series (A-D). I, II and III: respectively 1st, 2nd and 3rd instar larvae

Parameter	Series A		Series B		Series C		Series D	
	I	II-III	I	II-III	I	II-III	I	II-III
Average individual dry weight calculated per filter (µg)	1.81	1.56	1.88	1.36	1.93	1.42	1.81	2.08
	1.77	1.51	1.88	1.42	1.89	1.32	1.75	1.69
	1.75	1.46	1.92	1.33	1.71	1.44	1.87	1.52
	1.75	1.41	1.88	1.43	1.82	1.35	1.82	1.46
	1.64	1.59	1.75	1.45	1.91	1.49	1.82	1.73
	2.03	1.17	1.87	1.34	2.00	1.48	1.80	1.07
	1.84	1.50	1.95	1.46	2.00	1.30	2.01	1.65
	2.03	1.64	1.87	1.48	1.71	1.40	1.93	1.62
	1.61	1.48	1.91	1.41	1.70	1.40	2.20	1.51
	1.80	1.54	1.86	1.48	1.71	1.44	1.89	1.69
Average individual dry weight per series (µg)	1.80	1.48	1.88	1.42	1.84	1.41	1.89	1.6
(Coefficient of variation, %)	(8)	(9)	(3)	(4)	(7)	(5)	(7)	(16)
Ash content (%)	5.57	11.33	5.83	10.75			6.54	11.68
	5.87	11.38	5.77	11.14			6.53	11.76
	5.83	11.22	5.88	11.13			6.39	11.66
	5.81	10.67	5.90	11.11			6.43	—
	5.79	11.03	5.86	10.97			6.48	—
Average ash content (%)	5.77	11.13	5.84	11.02			6.47	11.7
(Coefficient of variation, %)	(2)	(3)	(1)	(2)			(1)	
Average individual ash-free dry weight (µg)	1.70	1.32	1.77	1.26			1.77	1.41
Caloric content/g ash-free weight (cal)	5,500	5,526	5,744	5,725			5,964	5,138
	5,804	5,616	5,666	5,347			5,688	5,273
	5,889	5,719	5,652	5,337			5,549	5,583
	5,698	5,541	5,877	5,563			6,020	—
	5,848	5,756	5,678	5,636			—	—
	—	5,573	5,689	5,713			—	—
Average caloric content	5,748	5,622	5,718	5,554			5,804	5,331
(Coëfficient of variation, %)	(3)	(2)	(1)	(3)			(4)	(4)
Average individual caloric content (µcal)	9.77	7.42	10.12	6.99			10.3	7.5

TABLE II

Biochemical composition of *Artemia salina* nauplii

Parameter	Instar I	Instar II-III	Decrease or increase in % from instar I to instars II-III
Individual dry weight (μg)	1.85	1.48	—20
Ash weight (%)	6.03	11.28	+88
Ash-free dry weight (μg)	1.75	1.33	—24
Caloric content/g ash-free dry weight (cal)	5.557	5.503	—4
Individual caloric content (μcal)	10.06	7.30	—27
Total lipid content (as % of the dry weight):			
Gravimetric method	19.3	13.7	—26
Colometric method	22.7	16.7	—26
Fatty acids (as % of the dry weight)	14.7	10.9	—26

TABLE III

Percentage of the fatty acids in *Artemia salina* nauplii

Fatty acids	Instar I	Instar II-III
Peaks not identified	15	14
16:0	11.4	11.9
16:1	5.7	5.7
18:0	5.2	6.4
18:1	32.2	33.9
18:2	7.1	6.7
18:3	23.9	21.2

Discussion

The literature data on the biochemical composition of *Artemia salina* nauplii from the San Francisco Bay- (California, U.S.A) and the Great Salt Lake- (Utah, U.S.A.) strains are summarized in Table IV. From these data it appears that there are considerable differences in the biochemical data from one author to another.

Paffenhöfer (1967) found that Utah-nauplii from the Great Salt Lake, show important changes in their biochemical composition, after a starvation period of 96 hr at 30 °C. Von Hentig (1971) reported significant differences in the individual dry weight, the caloric content, the protein, lipid and carbohydrate content of Utah nauplii hatched at 16 different temperature-salinity combinations. As in most cases, neither the conditions

TABLE IV

Literature data on the biochemical composition of *Artemia salina* nauplii from California and Utah

Reference	Individual weight (µg)	Pro-teins	Li-pids	Carbo-hydra-tes	Ash	Calories/g ash-free dry weight	Individual caloric content (µcal)
		(% of the dry weight)					
Great Sakt Lake, Utah							
Nauplii (hatched at 30 °C in seawater of 32 ‰ salinity) (Von Hentig, 1971)	1.92	41.6	23.1	22.7	6.56	5,893	0.010
Nauplii 24 hr old (at 20 °C) (Paffenhöfer, 1967)	1.58	—	—	—	9.81	5,929	0.003
Nauplii 48 hr old (at 20 °C) (Paffenhöfer, 1967)	1.42	—	—	—	11.49	5,627	0.007
Larvae of 2 mm length fed *Dunaliella* (Paffenhöfer, 1967)	—	—	—	—	11.68	5,855	—
Nauplii 2 days old (Katsutani; 1965)	—	—	15.2	—	9.6	—	—
San Francisco Bay, California							
Nauplii (Dutrieu, 1960)	2.87	42.5	23.2	—	—	6,600	—
Nauplii (as calculated by Von Hentig, 1971 from the results of Urbani, 1959)	1.50	—	—	—	—	—	0.008
Nauplii (Clegg, 1962)	1.93	—	—	—	—	—	—
Nauplii (Brick in Helfrich, 1973)	—	50.3	15.9	—	—	—	—
Nauplii (Coehn in Helfrich, 1973)	—	50.0	27.2	—	—	5,896	—
Larvae of 0.6 mm length (Dutrieu, 1960)	2.7	50.6	23.2	6.0	14.7	5,964	—
Larvae 6 days old (Brick in Helfrich, 1973)	—	59.7	7.0	—	—	—	—

in which the larvae were hatched, nor the developmental stage of the larvae at the moment of the analysis, nor the methods of analysis were mentioned, and a comparison with our results would be very speculative.

Since Remiche-Van der Wielen and Sorgeloos (in press) demonstrated that the hatching- and molting rate of *Artemia* nauplii are strongly temperature dependent, it is clear, from our biochemical analyses, that in order to always harvest larvae with the same biochemical composition, it is of paramount importance to standardize the hatching techniques as well as the consecutive treatment of the larvae, not at least with respect to the incubation temperature and to the time of harvest.

Considering the 20 % weight decrease between the first and next two instar stages, it is, from a practical point of view, clear that feeding predators with 2nd and 3rd stage *Artemia* larvae instead of with first instars, requires 20 % more larvae to offer the same food biomass.

This means not only a 20 % increase of the quantity of cysts to be hatched, but also a higher energy consumption of the predators which must chose and ingest a considerable higher number of (nutrionally poorer) *Artemia* larvae, to guarantee the same nutritional food uptake.

On the basis of the caloric content of the *Artemia* larvae, the fish larvae will have to take up 27 % more 2nd and 3rd instars than when fed 1st stage nauplii.

From the present results we tentatively postulate, that the nutritional value of the larvae decreases from the 1st to the 2nd and 3rd instar stage. This hypothesis corroborates the findings of Morris (1956) who stated : "... the yolk-rich quality of the early nauplius appears to be one of the primary attributes of this organism for feeding larvae of at least some marine fishes ; ... the (fish) larvae have not prospered in rearing trials where they fed only on *Artemia* nauplii which had used up their yolk".

Further biochemical analyses, complemented by feeding tests are needed, in order to check the hypothesis set forth and to evaluate the practicality of stocking and feeding unfed *Artemia* nauplii to predators, several days after hatching. (Jones, 1972 ; Tabb *et al.*, 1972 ; Salser and Mock, 1974).

In conclusion we should warn the researcher dealing with fundamental studies in aquaculture where *Artemia* nauplii are used as a food source, that the uncontrolled feeding of batches containing unknown mixtures of 1st, 2nd and 3rd instar nauplii, can greatly influence the results that are obtained.

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